

Supplementary Material

Biallelic, Selectable, Knock-in Targeting of CCR5 via CRISPR-Cas9 Mediated Homology Directed Repair Inhibits HIV-1 Replication

Stefan H. Scheller, Yasmine Rashad, Faye M. Saleh, Kurtis A. Willingham, Antonia Reilich, Dong Lin, Reza Izadpanah, Eckhard U. Alt, Stephen E. Braun

Table S1: Oligonucleotide sequences

Primer	Sequence (5'-3')	Function
sgRNA1	CTTTTATTATGCACAGGG	Single-guide cloning
sgRNA2	TAATAATTGATGTCATAGAT	Single guide cloning
sgRNA3	TGACATCAATTATTATACAT	Single guide cloning
sgRNA4	CTTCACATTGATTTTTGGC	Single guide cloning
LHA	AAGCTTGGATCCCCTAGGTTGACATC AATTATTATACATCGGTGAATG TGACATCAATTATTATACATCGGTGAAT GGGTATGATGCTTAGAACAG CAGACTATCTTCTAGGGTTGATGTCA TAGATTCCACTTGACAC	Cloning of the left homology arm for Gibson assembly, introduction of gRNA3 sequence and mutation of the NGG
RHA	ATGATTATCTTCTAGGGTTATTATTATA CATCCCAGCCCTGCC CCGATGTATAATAATTGATGTCAAGAG CTACTGCAATTATTCAAGGC GCATACGCGTATACTAGGTTCCGATGT ATAATAATTGATGTCAAGAGCT	Cloning of the right homology arm for Gibson assembly, introduction of gRNA3 sequence and mutation of the NGG

hU6-F	GAGGGCCTATTCATGATT	Sequencing of the pCas9-gRNA
Seq-RHA3`-junction (SeqM13)	CAGGAAACAGCTATGAC	Sequencing of the Donor Plasmids
Seq-LHA5`-junction	CCTAGCAAACCTGGGCACAAGC	Sequencing of the Donor Plasmids
T7EI	GCTTCATTCACTCCATGGTGTAT CAAAGTCCCCTGGCGGG	T7EI Assay
DWT	GCATTCATGGAGGGCAACTAAA GGCTGCGATTGCTTCACATT	qPCR
VKI left for	AGGCTTCCCGCATTCAAAATC	qPCR
VKI left rev Seq-LHA3`-junction	CCTAAATGCACAGCGACGGA	qPCR/ Sequencing of the Donor Plasmids
VKI right for Seq-RHA5`-junction	ACCGATAAAACACATGCGTCA	qPCR/ Sequencing of the Donor Plasmids
VKI right rev	CAGTGCGTCATCCCAAGAGT	qPCR
PuroR	AGAGGAAGTCTTCTAACATGCGGT AGAGTTCTTGCAGCTCGGTG	qPCR

Table S2. CRISPOR-Results

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# Sequence CTTTTTATTATGCACAGGGTGGAAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTACATCGGAGCCCTGCC  
AAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCTGCCTCCGCTACTCACTGGTGTTCATCTGGTTGTGGCAACATGCTG  
GTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGACATCTAC
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Genome GrCh37/hg19

PAM 20bp-NGG - SpCas9

Position chr3:46414382-46414597:+

Version CRISPOR 4.8, 2019-04-12T21:49:22CEST

Results http://crispor.org/crispor.py?batchId=ObBtkMaHaiicT2KjpKmN

#guidelid	targetSeq	mitSpecScore	offtargetCount	targetGenomeGeneLocus	Doench '16-Score	Moreno-Mateos-Score	Out-of-Frame-Score	Nomenclature in Study
3forw	CTTTTTATTATGCACAGGGTGG	58	249	exon:CCR5	51	35	68	(gRNA 1)
173rev	TCAGCCTTTGAGTTATCAGG	72	164	exon:CCR5	35	29	42	
133rev	TAATAATTGATGTCATAGATTGG	69	241	exon:CCR5	40	23	71	(gRNA 2)
61forw	TGACATCAATTATTATACATCGG	64	251	exon:CCR5	56	39	64	(gRNA 3)
167rev	TTTGCACTTATCAGGATGAGG	63	276	exon:CCR5	53	49	31	
67rev	CTTCACATTGATTTCGGCAGG	60	292	exon:CCR5	38	0	79	(gRNA 4)
127forw	CCTGCCCTCGCTACTCACTGG	58	196	exon:CCR5	47	56	60	
189forw	TCATCCGTATAACTGCAAAAGG	58	258	exon:CCR5	50	22	44	
111rev	AACACCAGTGAGTAGAGCGGAGG	56	100	exon:CCR5	62	58	62	
66rev	TTCACATTGATTTTGGCAGGG	52	410	exon:CCR5	45	22	79	
114rev	ATGAACACCAGTGAGTAGAGCGG	52	210	exon:CCR5	66	49	65	
140forw	TACTCACTGGTGTTCATCTTGG	51	159	exon:CCR5	30	52	57	
104rev	GTGAGTAGAGCGGAGGCAGGAGG	48	403	exon:CCR5	56	86	67	
160forw	TGGTTTGTGGCAACATGCTGG	43	249	exon:CCR5	44	34	33	
107rev	CCAGTGAGTAGAGCGGAGGCAGG	41	210	exon:CCR5	51	40	75	
149forw	GTGTTCATCTTGGTTTGTGGG	34	399	exon:CCR5	31	52	51	
148forw	GGTGGTACATCTTGGTTTGTGG	33	289	exon:CCR5	40	23	54	
71rev	TTTGCTCACATTGATTTCGG	32	687	exon:CCR5	18	29	73	
100rev	GTAGAGCGGAGGCAGGAGGCGG	23	791	exon:CCR5	47	66	68	
101rev	AGTAGAGCGGAGGCAGGAGGCGG	20	800	exon:CCR5	48	76	64	

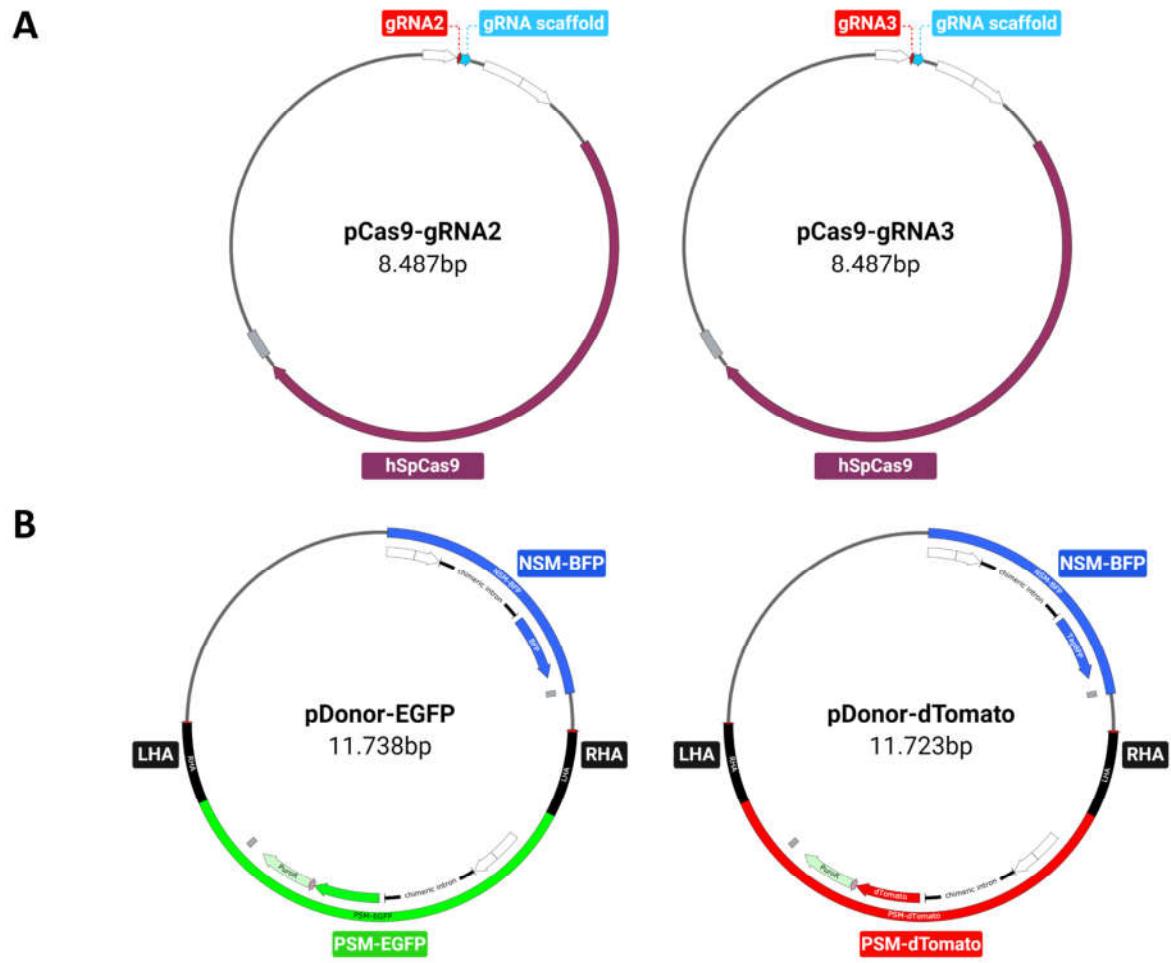


Fig. S1. Schematic diagram of the plasmids used in this study **A:** For dual targeting, two identical hSpCas9 expressing plasmids were equipped with two different gRNAs (gRNA2 or gRNA3). **B:** The donor plasmids consist of a BFP expression cassette as negative selection module (NSM-BFP) and a positive selection module encoding for EGFP in one donor (PSM-EGFP) and dTomato (PSM-dTomato) in the other, which is flanked by the same sequences homologous for the targeted region (LHA and RHA)

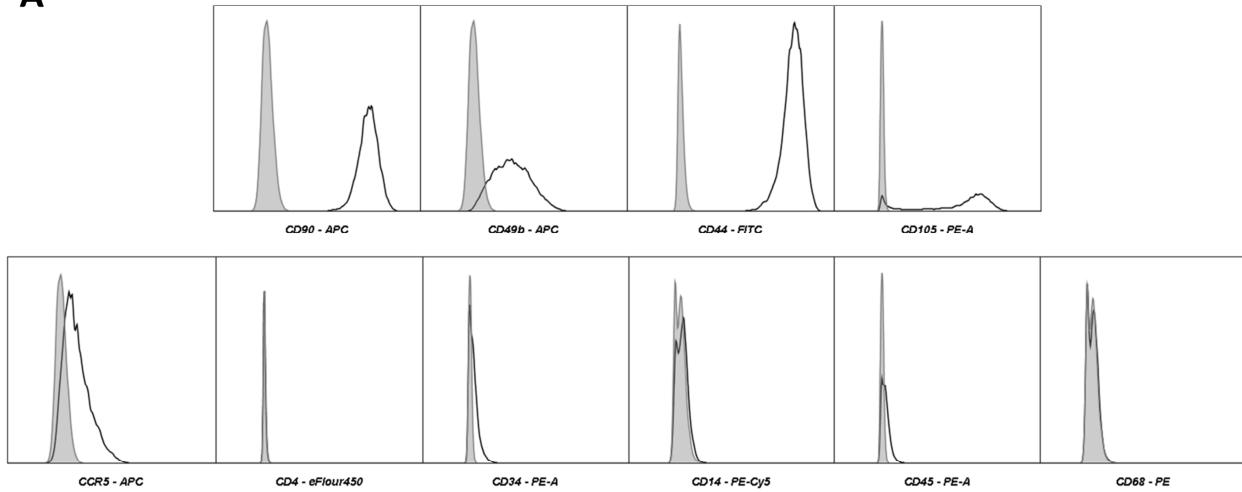
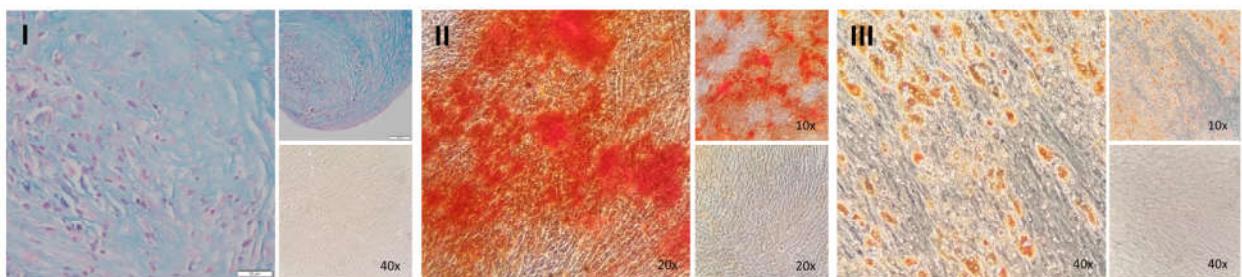
A**B**

Fig. S2. Characterization of ASC as a multipotent stem cell. **A:** Immunophenotypic analysis of cell surface profile of freshly isolated ASCs. Cells were stained with fluorescent marker labeled antibodies for CD90, CD49b, CD44, CD105, CCR5, CD4, CD34, CD14, CD45 and CD68. In the histogram the gray filled graph represents the signal of an unstained control, the black line the population is stained with the corresponding antibody. **B:** Multilineage differentiation potential of freshly isolated ASC. Isolated cells were capable of differentiating into (I) chondrogenic (extracellular proteoglycans appear blue in toluidine blue staining), (II) osteogenic (alizarin red staining marks calcium deposits), (III) adipogenic (intracellular lipid vesicles appear red using Oil Red O staining) lineage.

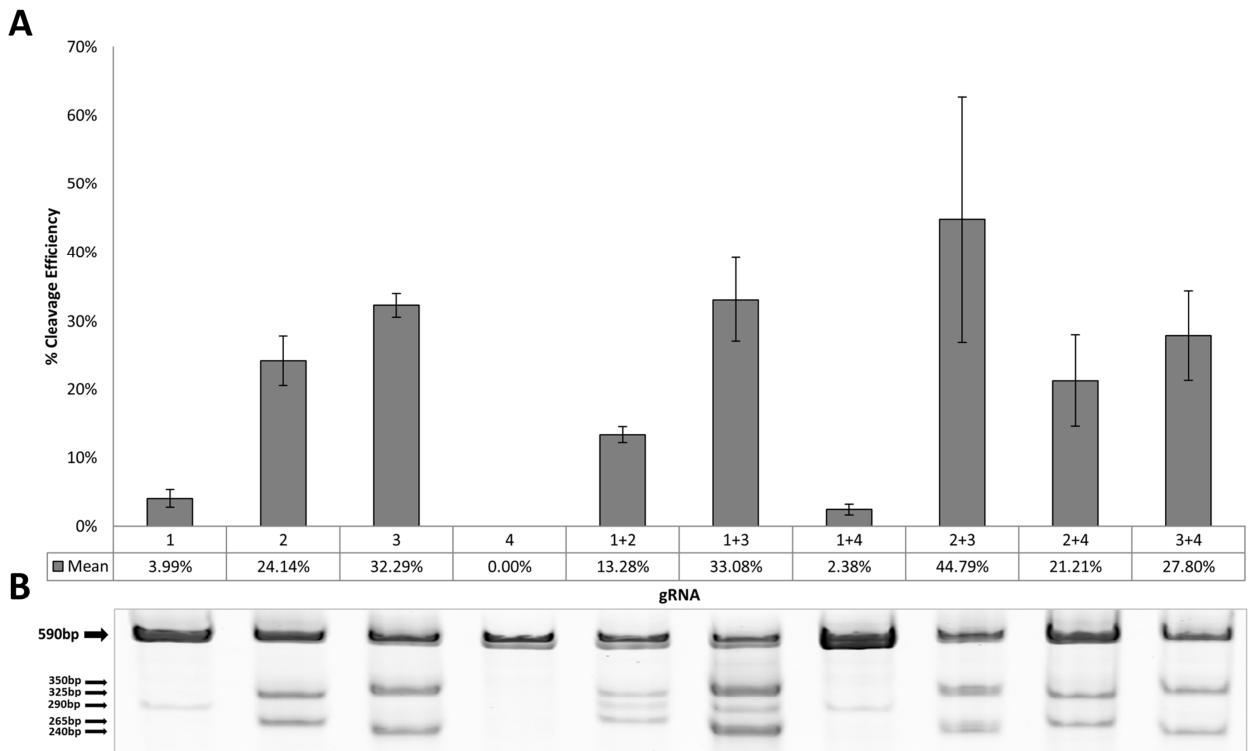


Fig. S3. Identification of the most efficient gRNA and their combinations. **A:** Four gRNAs (1-4) were cloned into Cas9 expressing Vectors and transfected into HEK 293FT using Lipofection. Additionally dual combinations of the gRNAs were transfected. The targeted site was amplified via PCR and a T7EI assay was performed. Gels were analyzed with ImageJ software and cleavage efficiency was calculated according to band intensity. Transfection and analysis were performed in triplicates. **B:** Representative of one of the triplicate T7EI-Assays visualized on TBE-Gels. Uncut or InDels not detected by the T7 Endonuclease are represented by a 590bp sized band. Bands of lower size represent mutational activity of the different gRNAs (gRNA1 => 290bp; gRNA 2 => 265,325bp; gRNA 3 => 240,350bp; gRNA 4 no bands detectable).

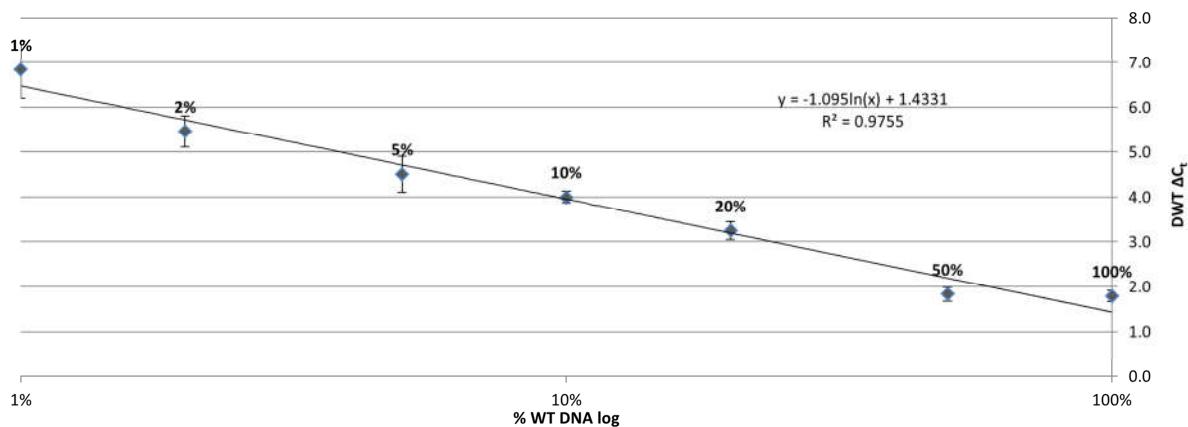
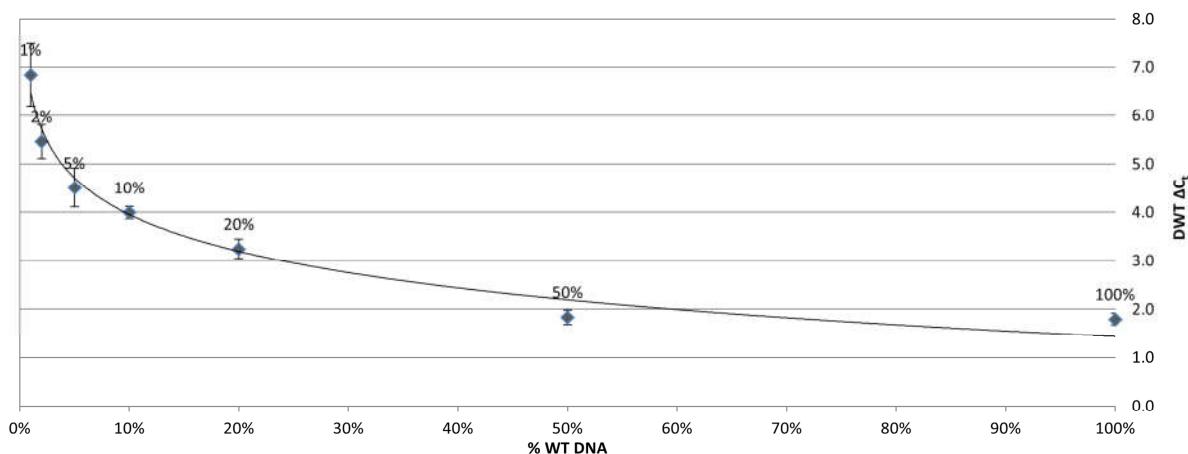
A**B**

Fig. S4. Standard Curve to determine the fraction of alleles not carrying a Knock-In (DWT) by linear regression from a DWT signal. Genomic DNA extracted from a dually positive clone with integration of the donor on both alleles and WT genomic DNA were mixed at ratios of 1, 2, 5, 10, 20, 50, and 100% WT DNA and subjected to qPCR for the DWT Amplicon. ΔC_t was calculated by normalisation with β -actin Ct value. The Standard Curve had to be normalized for every cell type analysis individually, by setting the WT DWT ΔC_t to 100% of alleles, not carrying a knock-in. **A:** The % of WT DNA is displayed in a logarithmic manner. **B:** The % of WT DNA is displayed in a linear manner. Due to the logarithmic nature of qPCR, the Ct Value is only sensitive for reflecting the % WT Alleles in the lower ranges. Small changes in the Ct signal reflect massive changes in the calculated % WT DNA in populations with an high fraction of WT alleles.

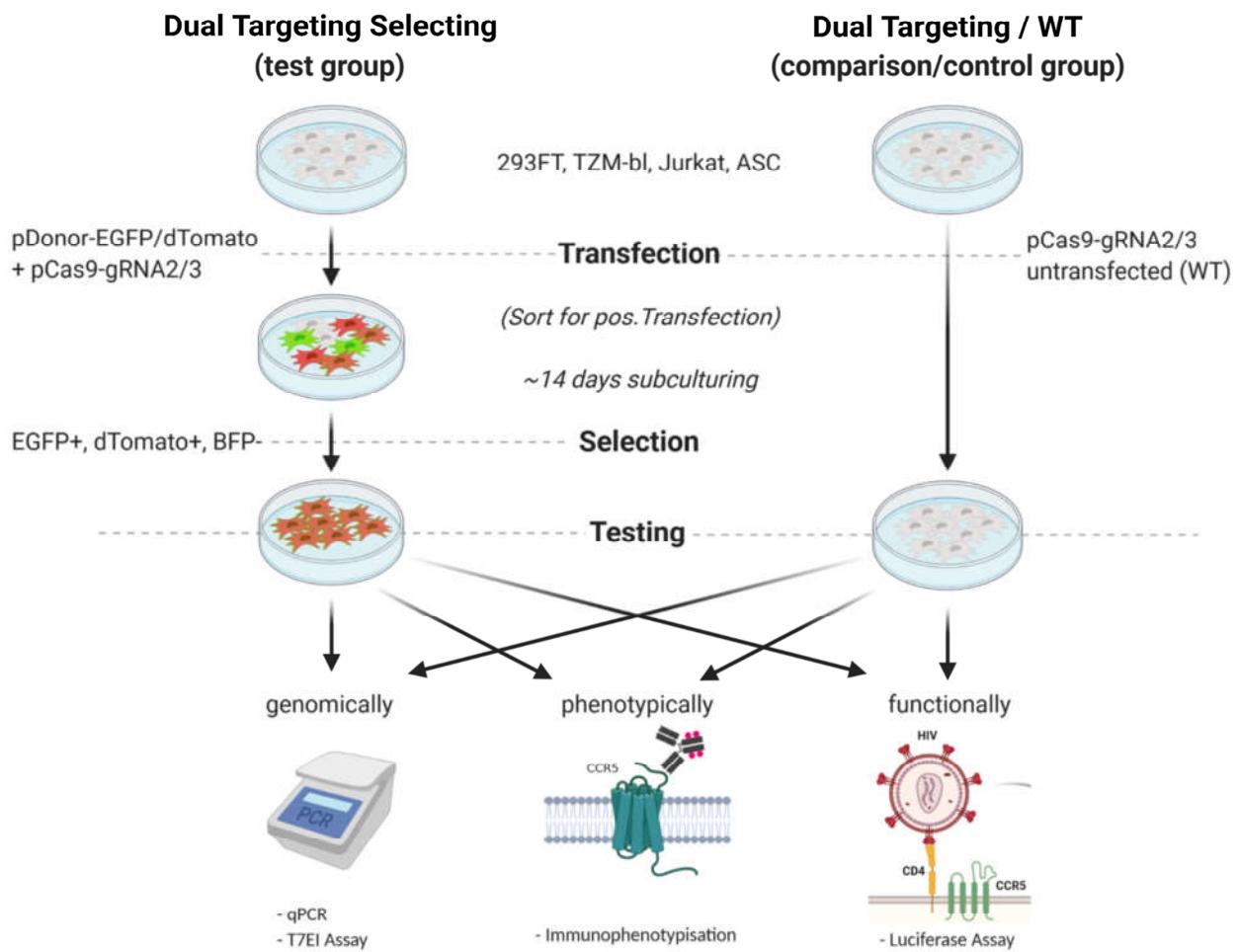


Fig. S5. Experimental Outline / Graphical Abstract